CHAPTER 28

Investigations of the Toxicokinetics of Hydrophobic Contaminants in the Zebra Mussel (*Dreissena polymorpha*)

Susan W. Fisher, Duane C. Gossiaux, Kathleen A. Bruner, and Peter F. Landrum

Physiological and toxicokinetic parameters for the zebra mussel, *Dreissena polymorpha*, were examined along with effects of feeding and temperature on selected measurements. Filtration rate for *Dreissena* was related to the algal concentration and ranged from 352 to 2651 mL/gDW/hr. There was a trend toward higher filtration rates in smaller mussels but the trend was insignificant. Oxygen consumption was inversely proportional to mussel size and directly proportional to temperature. Oxygen consumption ranged from 6.9 mgO_2/gDW/day at 4°C to 60.8 mgO_2/gDW/day at 23°C. Uptake clearance rates for contaminants from water exhibited similar relationships with temperature and mussel size and an additional direct proportionality with the lipophilicity of contaminants as represented by log K_{ow}. At 20°C, mean uptake clearances ranged from 428 to 1073 mL/gDW/hr across a range of compounds with log Kow values of 5.2–6.7. Efficiency for oxygen accumulation was much lower than that for contaminants, while filtration rate for a wide range of particle sizes was similar to uptake clearances for contaminants. Thus, it appears

that high filtration rates are not a result of oxygen requirements but rather food requirements, and thus dissolved contaminants are effectively accumulated. Elimination of contaminants was relatively slow with half-lives ranging from 41 to 173 hr for the range of contaminants studied. The presence of food complicates contaminant accumulation by sorbing contaminants and reducing their availability on a whole-water concentration basis while also increasing rates of contaminant elimination. Overall, high filtration rates, relatively high bioconcentration potential, and high fecundity of zebra mussels will probably affect cycling of contaminants in the Great Lakes.

INTRODUCTION

Polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) are consistently identified as Great Lakes priority pollutants because of their presence in detectable concentrations in water, biota, sediment, and suspended particles of the Great Lakes (Fitchko, 1986). The high lipid solubility of these chemicals facilitates sorption to particulate organic matter in water and sediment, and they also partition readily into the lipid tissue of aquatic organisms. Although sorption to sediment is generally thought to reduce biological availability and thus decrease exposure to organisms in the water column, it is clear that benthic invertebrates — which ingest and process contaminated sediment — may have a major role in transferring sorbed contaminants to pelagic food chains (Neff, 1979; D'Itri and Kamrin, 1983; Landrum, 1988).

Less studied, but potentially as important, is the role of zebra mussels in contaminant cycling in the Great Lakes. High filtering rates increase the probability that populations of zebra mussels will be exposed to a wide range of pollutants including hydrophobic contaminants such as PCBs and PAHs. In addition, mussels actively filter contaminated particles and algae. If algae are not ingested by mussels, they are eliminated as pseudofeces and deposited on the bottom. The biological availability of contaminants sorbed to pseudofeces has not been investigated but may serve as a reservoir from which feeding and burrowing activities of benthic invertebrates can recycle PCBs and PAHs. In order to accurately assess the impact of zebra mussels on the Great Lakes, it is imperative to understand the extent to which such contaminants can be accumulated in mussels.

The purpose of the current study was to examine the basic toxicokinetics of zebra mussels in the Great Lakes. Specific objectives were to: (1) determine accumulation and loss kinetics of selected environmental pollutants; (2) determine filtering rate as a function of particle size and respiration rates as a function of animal mass and environmental temperature; (3) examine the influence of size and temperature on toxicokinetics; (4) correlate kinetic parameters with log Kow; and, (5) perform an interlaboratory comparison of toxicokinetic parameters in two mussel populations. This information will

increase our knowledge of the potential impact of zebra mussels on contaminant cycling in the Great Lakes and North America.

METHODS

Zebra mussels used in these studies were collected from two geographically distinct locations. Individuals used in experiments at the Great Lakes Environmental Research Laboratory (GLERL) were collected at a site in Lake St. Clair (42°20′00″ N and 82°47′30″ W) with a water depth of 5 m. Mussels were collected using an epibenthic sled, cleaned with lake water, and placed in a cooler containing lake water and transported to the laboratory. In the laboratory, mussels were transferred to aerated aquariums. Individuals used in experiments at Ohio State University (OSU) were collected at a site in Lake Erie with a water depth of 3 m, located 15 m offshore from Catawba Point, Marblehead, OH. In general, mussels were removed from rocks, rinsed with lake water, and placed in coolers with aeration for transport to holding facilities. Adult mussels were maintained in 208-L Plexiglas™ aquaria filled with Lake St. Clair water (GLERL stock) or aged, aerated tap water (OSU stock). Initial collections of both experimental stocks of mussels were made in July 1990 when water temperature was between 21 and 23°C. Mussels were maintained in the laboratory at room temperature and fed a diet of pelleted *Chlorella* at the rate of approximately 3.3 g *Chlorella* per 1000 mussels per day. Water was monitored daily for ammonia buildup and changed completely every 2–3 days. Mean (±S.D.) wet weight of the Lake St. Clair mussels used in experiments was 78.7 ± 29.6 mg wet tissue (n = 74, range 30.1–156.7 mg) and mean length was 17.5 ± 2.9 mm (n = 74, range 11–23 mm); mean (±S.D.) wet weight of Lake Erie mussels used in the experiments was 96.8 ± 21.6 mg tissue (n = 80, range 61.1–137.0 mg) and mean length was 21.2 ± 1.51 mm (n = 80, range 20.0–25.0 mm).

Chemicals used in kinetic experiments were ^{14}C-labeled *p,p′*-DDT (12.2 mCi/mMol, OSU; 13.5 mCi/mMol, GLERL); 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP, 20.0 mCi/mMol, OSU; 17.6 mCi/mMol, GLERL); [^{3}H]benzo(*a*)pyrene (BaP, 33.1 Ci/mMol); 3,4,3′,4′-tetrachlorobiphenyl (TCBP, 15.7 mCi/mMol); and [^{3}H]pyrene (Pyr, 34 Ci/mMol). Radiopurity of compounds was >98%, as confirmed by thin-layer chromatography (TLC) on silica gel plates using hexane:benzene (8:2 V:V) and liquid scintillation counting (LSC) prior to use in kinetic experiments (Landrum, 1988).

Uptake Clearance and Elimination Rate Constants

Several days prior to initiation of experiments, adult zebra mussels were removed from stock cultures by severing their byssal threads with a razor

blade. Individual mussels were then allowed to reattach to glass microscope slides (OSU) or glass petri dishes (GLERL) in culture water contained in 38-L aquariums. Only mussels which secreted new byssal threads and reattached within 24–48 hr were used in kinetic experiments.

To initiate uptake clearance experiments, ^{14}C- or ^{3}H-labeled compounds were added in bulk to 15–20 L of experimental water (Lake St. Clair water filtered through Gelman AE glass fiber filters was used in GLERL experiments, unfiltered Lake Erie water was used in OSU experiments) using 100 μL of acetone or methanol carrier. Bulk experimental water was allowed to equilibrate for 1 hr (GLERL) or 24 hr (OSU). Contaminated water was then divided among treatment vessels. Contaminant concentrations varied from 4 pg/L to 1 ng/L. Mussels in GLERL experiments were individually exposed to each contaminant in 600-mL beakers filled with 500 mL of treated lake water to afford a mass-balance analysis. Mussels used in OSU experiments were exposed to contaminants in one of three 40-L aquariums at densities of 3/L during the uptake phase. In both laboratories, uptake experiments were conducted for 6 hr and samples of zebra mussels were withdrawn for determination of total radioactivity after 0.5, 1.5, 2.0, 3.0, and 6.0 hr of exposure. Water samples of 2 mL (GLERL) or 1 mL (OSU) were withdrawn initially and at each sampling time. A minimum of three replicates were analyzed at each sampling time.

Mussels which had been simultaneously exposed to contaminants along with mussels that were used in uptake experiments were transferred to clean, uncontaminated water in order to measure elimination of each compound. In GLERL experiments, elimination was measured in static systems filled with lake water. The elimination water was renewed daily. In OSU experiments, mussels were placed in flow-through systems through which Lake Erie water was added with a gravity feed at a flow rate of 500 mL/hr. This flow rate was sufficient to keep eliminated contaminants below detectable limits in the water. Mussels were withdrawn from elimination experiments at specific time periods for liquid scintillation counting along with water samples to confirm that secondary uptake was not occurring. For OSU experiments, sampling times during the elimination phase were 0, 1, 3, 7, 17, 24, and 488 hr. For GLERL experiments, samples were withdrawn at 0, 12, 24, 96, 144, 192, 264, and 360 hr. A minimum of three replicates were used for each sample time.

Mussels were analyzed for total radioactivity immediately after removal from uptake and elimination experiments. Individuals were blotted dry, weighted, and measured (i.e., shell length). Soft tissue was then dissected from the shell and each component weighed separately. Shells and soft tissues were placed separately in 20-mL glass scintillation vials to which 5 mL of scintillation cocktail (dioxane:naphthalene: PPO, 1000/100/5; OSU) or 12 mL of RPI 3a70b scintillation cocktail (GLERL) were added. Water samples were

pipetted directly into vials containing scintillation fluid. Total radioactivity was measured by liquid scintillation counting. Each vial was counted for a minimum of 5 min in a Beckman LS 6000IC scintillation counter with automatic quench control (OSU) or 10 min on an LKB 1217 Rack Beta scintillation counter (GLERL). Samples were corrected for quench using the external standards ratio method after subtracting background. The efficiency for measuring radiolabeled compounds after direct extraction by scintillation cocktail was examined by comparing organisms with the same exposure where the tissue had been predigested with Protosol. Both methods yielded equivalent tissue concentrations.

The standard temperature at which uptake and elimination experiments were run was 20°C. However, temperature was altered in a series of experiments conducted at GLERL to address effects of changing temperature on toxicokinetic parameters. In these experiments, uptake and elimination experiments for HCBP and BaP were conducted at 4, 12, and 20°C. Organisms examined at lower temperatures were acclimated from 20°C to lower temperatures at 2°C/day and held at experimental temperatures for a minimum of 24 hr prior to use. In addition to temperature experiments, effects of feeding on accumulation and elimination were evaluated for HCBP at OSU. Accumulation of HCBP was measured when mussels were exposed to contaminated water without food, to contaminated water in the presence of uncontaminated food, and to clean water with [^{14}C]HCBP-contaminated *Chlorella*. The alga was contaminated by adding radiolabeled HCBP to a slurry consisting of 12 g dried *Chlorella* in 120 mL of distilled water. The slurry was mixed on a rotary shaker for 48 hr after which a subsample was centrifuged at high speed for 10 min in a clinical centrifuge. The supernatant and pellet were then analyzed for total radioactivity with the finding that less than 1% of total HCBP was in the aqueous phase. Zebra mussels were exposed to contaminated *Chlorella* in static systems using a constant *Chlorella* drip. Elimination was measured in flow-through systems as previously described.

Respiration Rate

Oxygen consumption and clearance of oxygen from water was measured at GLERL as a function of temperature. In these experiments, one mussel (in replicates of three) was placed in a 60-mL BOD bottle with filtered lake water under yellow light (lambda $>$ 500 nm) for 6 hr. Initial and final samples of oxygen content of the water were determined as described by Grasshoff (1983). Oxygen consumption was measured for mussels held at 4, 10, 15, and 23°C. For each temperature, oxygen consumption was measured both in the absence and presence of contaminants.

Filtration Rate

The ability of adult zebra mussels to remove particles from the water was assessed as a function of particle size. To measure filtration rate, a stock solution of 8 mg, Sun *Chlorella* in 50-mL lake water was prepared in a 100-mL beaker. The solution was sonicated to break up any large clumps of *Chlorella*. This solution was added to 1.5 L of lake water in a volumetric flask and inverted several times to suspend fine material. The average concentration of *Chlorella* particles used in filtration studies was 45,110 particles per milliliter with a size range of 2- to 20-μm diameter. A portion of the solution (600 mL) was poured into each of two 1-L beakers. Wire screen supports were suspended approximately one quarter of the depth of the beaker, and the solution was stirred on a water-driven magnetic stirring plate with a magnetic stir bar. A 20-mL water sample was withdrawn from each of the duplicate beakers for determination of particle size and concentration. One zebra mussel was then placed on each wire screen and monitored for filtering activity. After 3 hr from initial opening, the mussel was removed and a second 20-mL sample was withdrawn. Mussels were measured for length, and the tissue removed and weighed. The size and concentration of particles were determined with a TA II Coulter Counter using the method of Sheldon and Parsons (1967). The aperture setting used for particle size determination was 140 μm. Filtration rates (particle clearance) were calculated from the following equation (Vanderpleog et al., 1982):

$$F = (V \ln(C/Z))/(t\ n)$$

F = filtering rate
V = volume of experimental container
C = concentration of algae at the end of experiment
Z = concentration of algae at the beginning of experiment
t = experimental duration
n = number of animals in experimental chamber

Clearance was calculated for 11 particle size classes ranging from mean spherical diameters of 2.0–20.0 μm.

Wet vs Dry Weight Ratio

Wet and dry weights were measured by first weighing the soft tissues wet and then drying them to a constant dry weight at 60°C. Data from the respiration and uptake experiments were thus expressed as a function of total weight, wet weight, and dry weight.

Mass Balance Model

Kinetic processes of accumulation and loss can be modeled through either concentration-based or mass balance models depending on experimental configurations. In the case of studies performed at GLERL, the experimental design dictates that mass balance models be employed. The basic assumptions employed in this model are that the mass of contaminant does not change in the system and that no biotransformation of contaminants takes place. Biotransformation potential of these organisms was examined once and no biotransformation was found for a 6-hr exposure (Landrum, Unpublished data). In most uses of the mass balance model, contaminants are assumed to partition between the organism and the water. This produces the following equation:

$$\frac{dQ_a}{dt} = k_1 Q_w - k_d Q_a \qquad (1)$$

Then, assuming mass balance in the system:

$$A = Q_w + Q_a \qquad (2)$$

Where Q_a is the quantity of contaminant in the animal (ng), k_1 is the conditional uptake rate constant (per hr), Q_w is the quantity of contaminant in the water (ng), k_d is the conditional elimination rate constant (per hr), t is time (hr), and A is the total amount of compound in the system. In the present study, sorption to walls of experimental vessels and to shells may have contributed to overall mass balance. However, sorption to these two items was small, being only 1.6 ± 0.2% for the vessel and 0.6 ± 0.5% for shells of the total BaP in the system. Similar fractions were found for the other contaminants examined. Further, the amount of sorption was essentially constant throughout all experiments. Thus, sorption was assumed to occur with the addition of water to the beaker and mussels to the water, and would not contribute significantly to the mass balance. As a result, both sorption to glassware and shells were not incorporated into the model equation. Because elimination of compounds is not significant over the course of uptake experiments, the elimination term can be removed from the equation and the calculation simplified to the following integrated initial rates equation:

$$k_1 = (-\ln (1 - Q_a/A))/t \qquad (3)$$

This conditional rate constant is a system dependent value and must be converted to a system independent clearance (k_u) by the following equation:

$$k_u = k_1 \text{ (Volume of water/Wet mass of tissue)} \qquad (4)$$

k_u describes the volume of water scavenged of contaminant per amount of organism per time and has units of milliliters per gram per hour. This coefficient is conditional on the environmental conditions under which measurements are made. This calculation ignores the shell and water inside the shell as being part of the organism.

The model for determining accumulation in OSU experiments was simplified by the experimental design chosen. Because the volume of water employed for exposure was large compared to the total mass of organisms, the mass balance assumption was not required since the concentration of contaminants in the water did not change significantly over the period of the experiment ($p < 0.05$). Thus, the following equation could be employed:

$$\frac{dC_a}{dt} = k_u C_w - k_d C_a \quad (5)$$

where C_a is the concentration in the animal (ng/g), k_u is the uptake clearance (mL/g/hr), C_w is the concentration in the water (ng/mL), and all the remainder of the terms are the same as defined previously. When the initial rates assumptions of no biotransformation and no elimination over the course of the uptake exposure are employed, the equation simplifies:

$$\frac{dC_a}{dt} = k_u C_w \quad (6)$$

which integrates to:

$$C_a = k_u C_w t \quad (7)$$

The elimination data were fit to a first order elimination model for both GLERL and OSU experiments.

$$\frac{dC_a}{dt} = k_d C_a \quad (8)$$

Integration of the above yields:

$$\ln C_a = \ln C_a^o - k_d t \quad (9)$$

where C_a^o is the initial organism concentration (ng/g) at the beginning of the elimination experiment, and all the rest of the terms are as previously defined.

Bioconcentration factors were calculated from uptake and elimination rate constants:

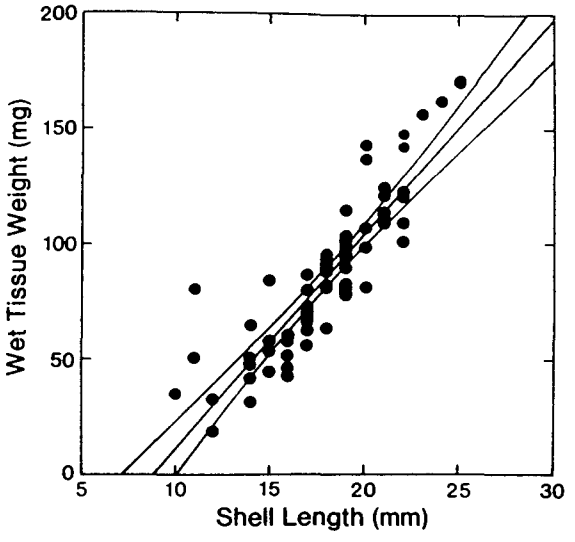

Figure 1. Relationship between wet tissue weight (WW in mg) and shell length (L in mm) in *Dreissena polymorpha*. WW = 9.39(±0.57) L − 85.5, r^2 = 0.764, $p < 0.001$, n = 85 (value in parentheses is ±S.E.).

$$BCF = k_u/k_d \tag{10}$$

Elimination half-lives were calculated directly from k_d values:

$$t_{(1/2)} = \frac{0.693}{k_d} \tag{11}$$

Multiple regressions were conducted for various toxicokinetic parameters as a function of temperature, organism mass, and/or physical parameters such as log K_{ow} using the Systat multiple regression program (Wilkinson, 1988). Analysis of variance was performed on regression parameters.

RESULTS

Physiological Characteristics

Regression analysis of shell length and wet tissue weight of zebra mussels in the GLERL experiments indicated that length was positively correlated with weight (Figure 1). In addition, the dry to wet tissue ratio was 0.131 ± 0.025 (mean ± S.D., n = 12) and wet tissue weight accounted for 16.2 ± 2.6% (mean ± S.D., n = 18) of the total (i.e., tissue and shell) weight, while 54.5 ± 2.6% (mean ± S.D., n = 18) of the total weight was water.

The mean filtering rate for all particle sizes depended linearly on particle concentration: filtering rate (mL/gWW/hr) = 21.8 (±6.1) Cp (mgDW/L) +

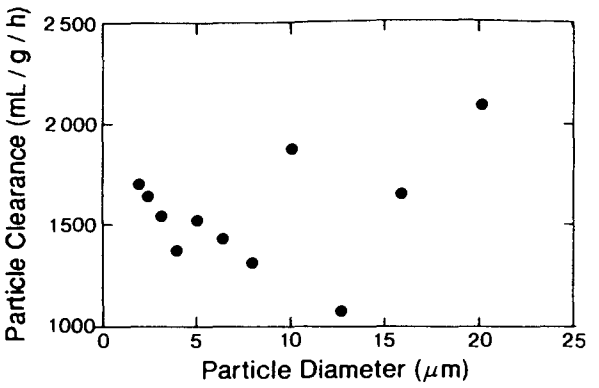

Figure 2. Clearance of particulate material from water by actively filtering adult *Dreissena polymorpha* as a function of mean particle size (μm).

TABLE 1. Mean (±S.E.) Oxygen Consumption and Clearance Rates of *Dreissena polymorpha* as a Function of Temperature

Temperature (°C)	Oxygen Consumption[a] ($mgO_2/g/d$)	Clearance of O_2[b] (mL/g/h)
23	60.8 ± 6.1 (n = 14)	40.8 ± 4.1
15	39.6 ± 4.9 (n = 29)	21.4 ± 2.6
10	19.5 ± 0.8 (n = 14)	9.5 ± 0.4
4	6.9 ± 0.7 (n = 15)	3.1 ± 0.3

[a] A Q_{10} value of 2.14 was calculated on a dry weight basis.
[b] Oxygen clearance was calculated on a wet weight basis.

288.7 (±237.8) ($r^2 = 0.52$, $p < 0.01$, n = 12), where Cp is the particle concentration. The range of filtering rates was 352–2651 mL/gWW/hr. Smaller mussels tended to have higher filtering rates than larger mussels. Filtration rates were not related to particle size over a size range of 2.0–20.0 μm in mean spherical diameter (Figure 2).

Oxygen consumption varied significantly ($p < 0.001$) with temperature from a low of 6.9 mgO_2/gDW/day at 4°C to a high of 60.0 mgO_2/gDW/day at 23°C (Table 1). From these data, a Q_{10} value of 2.14 of oxygen consumption was determined. When the weight of mussels was factored into the relationship between temperature and oxygen consumption in a multiple regression analysis, these two variables together explained 84.6% of the variation in oxygen consumption (Figure 3). As the weight of mussels increased, the rate of oxygen consumption declined significantly. Thus, the highest rate of oxygen uptake (i.e., clearance from the water) was measured in relatively small mussels (5–10 mgDW per mussel) at 23°C, and the lowest clearance of oxygen from water occurred in larger mussels (15–30 mgDW per mussel) at 4°C.

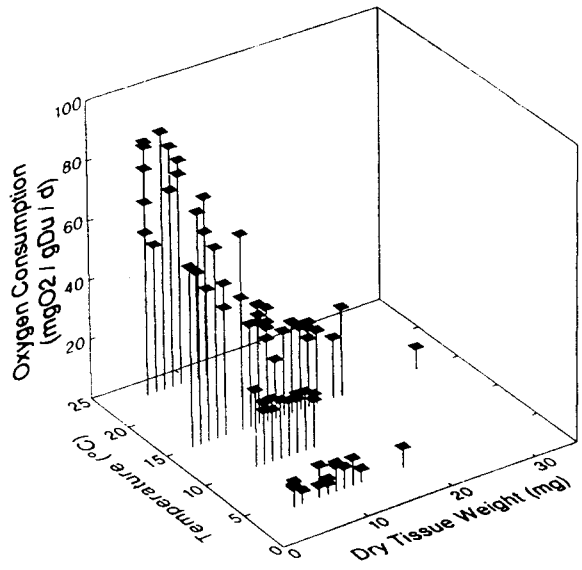

Figure 3. Oxygen consumption (R in mgO^2/gDW/day) as a function of temperature (T in °C) and dry weight (DW in mg) in *Dreissena polymorpha*. R = 9.5 (±3.7) − 1.71 (±0.24) gDW + 3.2 (±0.2) T; r^2 = 0.841, p <0.001, n = 72 (value in parentheses is ±S.E.).

TABLE 2. Toxicokinetic Parameters for Selected Organic Contaminants for *Dreissena polymorpha* in GLERL and OSU Experiments

Experiment	Compound	k_u[a] (mL/g/hr)	k_d[a] (/hr)	BCF	$t_{1/2}$ (hr)	K_{ow}
GLERL	B(a)P (n = 36)	838 (348)	0.009 (0.002)	76,182	63	5.98
GLERL	HCBP (n = 22)	1073 (407)	0.004 (0.002)	268,250	173	6.77
GLERL	DDT (n = 16)	736 (354)	0.0068 (0.001)	108,235	102	6.19
GLERL	TCBP (n = 14)	796 (317)	0.0169 (0.002)	47,100	41	5.95
GLERL	Pyrene (n = 16)	428 (202)	0.0096 (0.001)	44,583	72	5.2
OSU	HCBP (n = 30)	167 (81)	0.004 (0.0001)	41,750	173	6.77
OSU	DDT (n = 30)	124 (126)	0.008 (0.0002)	53,000	87	6.19

[a] Values given are means with standard deviations in parentheses.

Uptake and Elimination of Xenobiotics

For all contaminants studied, accumulation was rapid with significant uptake of each contaminant taking place within the uptake clearance phase of the experiment (Table 2). For the GLERL experiments, uptake clearance rates varied from a high of 1073 mL/gWW/hr for HCBP to a low of 428 mL/gWW/hr for relatively water soluble pyrene. Elimination rate constants, in

TABLE 3. Effects of Temperature on Accumulation and Elimination of BaP and HCBP in *Dreissena polymorpha* in GLERL experiments

Compound	T (°C)	k_u[a] (mL/g/hr)	k_d[a] (/hr)	BCF	$t_{1/2}$ (hr)
BaP	4 (n = 12)	415 (149)	0.0021 (0.001)	197,619	330
	12 (n = 16)	514 (225)	0.006 (0.001)	85,666	115
	20 (n = 12)	882 (403)	0.009 (0.002)	98,000	77
HCBP	4 (n = 15)	564 (280)	0.001 (0.0005)	564,000	693
	12 (n = 16)	715 (280)	0.004 (0.001)	178,750	138
	20 (n = 12)	1048 (459)	0.0039 (0.002)	268,718	175

[a] Values given are means with standard deviations in parentheses.

contrast, were low, indicating preferential retention by tissues and were monophasic for all compounds. BCF values calculated from GLERL experiments demonstrated that substantial residues of hydrophobic xenobiotics can be expected in environmental settings. Furthermore, kinetic parameters provide insight into the processes which determine body burden and which appear to vary by compound. For example, the high uptake clearance of TCBP (796 mL/gWW/hr) was compensated by a comparatively high elimination rate constant (0.0169/hr). Thus, the BCF value for TCBP was relatively low at 47,100. In contrast, the uptake clearance rate for pyrene was the lowest of all compounds examined in GLERL experiments (428 mL/gWW/hr), but the low elimination rate constant (0.0096/hr) rendered a BCF value of 44,583, which was comparable to that of TCBP.

The uptake clearance rate for HCBP in OSU experiments was very low compared to that determined in GLERL experiments (Table 2). However, uptake clearance rates for DDT were similar between the two sets of experiments as were elimination rate constants for both chemicals. The low k_u found in OSU experiments for HCBP resulted in a depressed BCF value relative to GLERL results. However, because elimination rate constants for both DDT and HCBP were similar for both data sets, the $t_{1/2}$ determined for these chemicals were comparable for both OSU and GLERL experiments.

For two chemicals, HCBP and BaP, effects of changing temperature were measured on toxicokinetic parameters in GLERL experiments. Uptake clearance rates for both HCBP and BaP increased significantly as temperature increased from 4 to 23°C (Table 3). Elimination rate constants likewise increased in response to increasing temperature. The net result of both changes was to cause a significant reduction in the projected BCF values and a similar depression in $t_{1/2}$ values as temperature increased. These trends were clearly evident for BaP. However, the elimination rate constant for HCBP did not

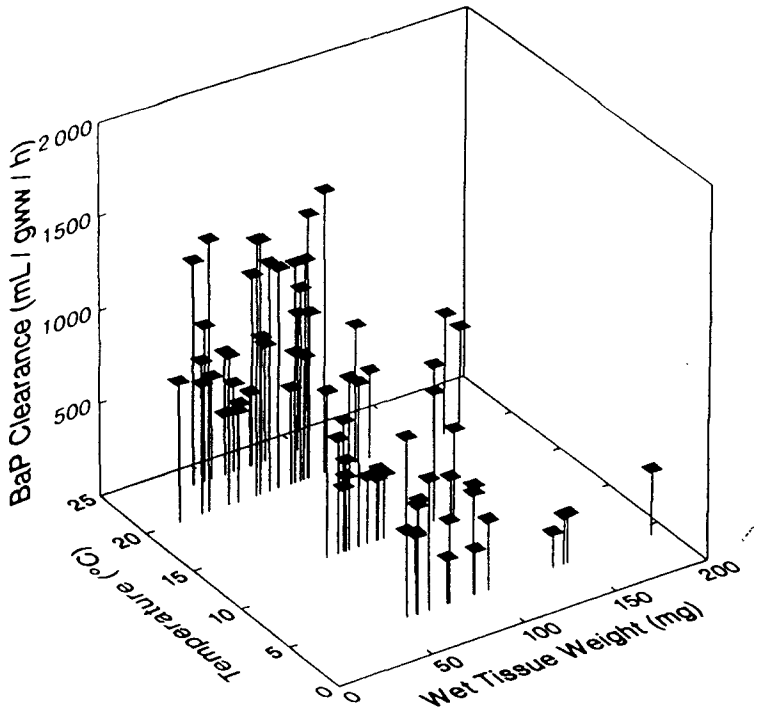

Figure 4. Relationship between uptake clearance rate for BaP (k_{uBaP} in mL/gWW/hr), temperature (T in °C), and wet tissue biomass (WW in mg) in *Dreissena polymorpha*. k_{uBaP} = 532 (±141) − 2.2 (±1.1) WW + 20.9 (±5.2) T; r^2 = 0.264, p <0.001, n = 68 (value in parentheses is ±S.E.).

change appreciably when water temperature was increased from 12 to 20°C. Thus, BCF values and $t_{1/2}$ did not increase as a function of elevated temperature for HCBP.

Multiple Regression Analyses

When the mass of a mussel was introduced as a variable in addition to temperature in kinetic experiments, a negative relationship occurred between mass and uptake clearance rate of BaP (Figure 4) and HCBP (Figure 5). In the case of BaP, temperature and mass accounted for only 26.4% of the total variation in k_u with temperature being the more important of the two variables. For HCBP, the combination of temperature and mass accounted for 38% of the variation in k_u. The rather low r^2 values indicate that other factors are important in determining uptake clearance rates in addition to temperature and mass.

For the GLERL experiments, uptake clearance rates (Figure 6), elimination rate constants (Figure 7), and BCF values (Figure 8) were examined as a function of both temperature and log K_{ow} in multiple regression analyses. Uptake clearance was significantly correlated with temperature and K_{ow} (Figure 6); the two independent variables contributed equally to changes in uptake

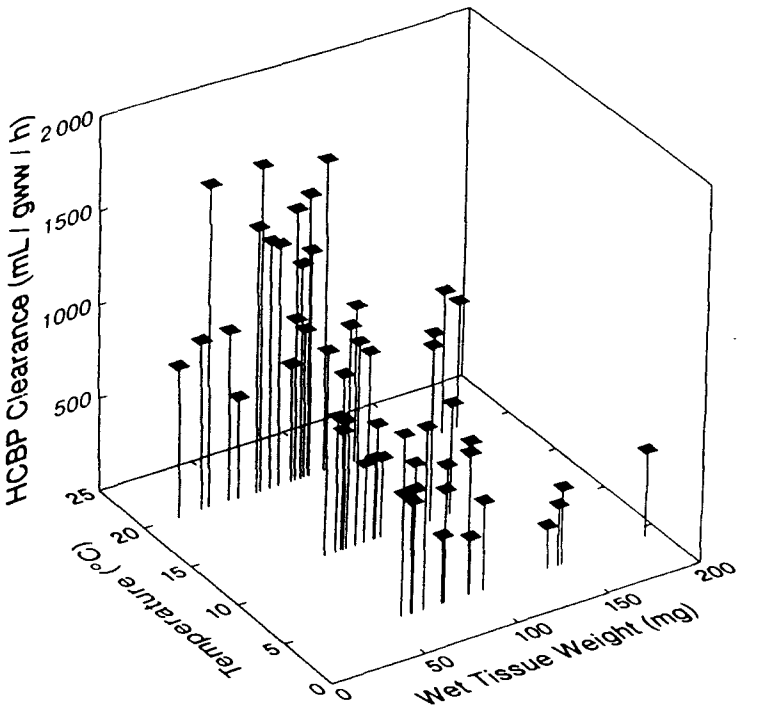

Figure 5. Relationship between uptake clearance rate for HCBP (k_{UHCBP} in mL/gWW/hr), temperature (T in °C), and wet tissue biomass (WW in mg) in *Dreissena polymorpha*. k_{UHCBP} = 738 (±153) − 3.6 (±1.2) WW + 29.7 (±6.5) T; r^2 = 0.38, p <0.001, n = 54 (value in parentheses is ±S.E.).

clearance rates and explained 75.8% of the variability in k_u. Uptake clearance rates increased significantly as temperature and contaminant lipophilicity increased. Elimination rate constants were likewise responsive to changes in temperature and K_{ow} although the latter was negatively correlated with elimination (Figure 7). Finally, BCF, which is a function of both uptake clearance and elimination rate constants, showed a significant negative relationship with temperature and a positive relationship with log K_{ow} (Figure 8). Both of these independent variables contributed equally to variation in the BCF.

Influence of Feeding and Exposure Route on Kinetics

Uptake clearance of HCBP in OSU experiments declined by a factor of 3 when uncontaminated *Chlorella* was added to the water as a food source (Table 4), while the elimination rate constant increased by a factor of 13. The accumulation of HCBP directly from algae in uncontaminated water was also measured for HCBP. In this case, K_u and K_d values of 7 mL/g/hr and 0.030/hr, respectively, were estimated. Bioconcentration factors and $t_{1/2}$ values varied as the route of exposure changed, with highest values occurring when chemicals were accumulated directly from water and lowest values occurring when assimilation from algae was required.

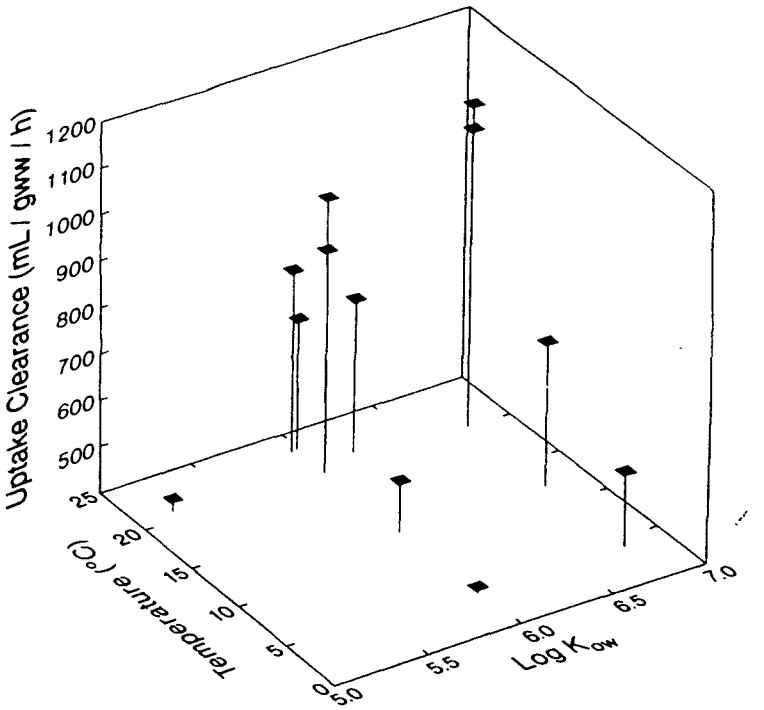

Figure 6. Relationship between mean uptake clearance rate (k_u in mL/gWW/hr) for a series of hydrophobic contaminants, temperature (T in °C), and log octanol:water partition coefficient (log K_{ow}) in *Dreissena polymorpha*. k_u = −1819 (±543) + 344 (±81) log K_{ow} + 25.7 (±5.7) T; r^2 = 0.71, p = 0.002, n = 12 (value in parentheses is ±S.E.).

DISCUSSION

Physiological Parameters

Results of filtering experiments suggest that small zebra mussels may have higher filtering rates than large mussels. Indeed, although the regression between mussel size and filtering rates was not significant (probably because of the small sample size), both respiration and contaminant clearance were correlated with size. For the marine mussel *Mytilus*, filtering rates are reported to be inversely, correlated with size (Vahl, 1973; Bayne and Widdows, 1978).

Although there are numerous reports of selective filtering of zebra mussels in response to food quality and particle size (Lee et al., 1972; Morton, 1971), selective filtering was not apparent in the present studies (Figure 2). However, the range of particle size employed in other studies was wider than the present study, ranging from a few to several hundred microns. An optimum particle size for ingestion by zebra mussels has been identified in the range of 15–40 μm (Ten Winkel and Davids, 1982). Thus, the particle size range used in this study was probably too narrow to permit evaluation of the relationship between particle size and filtering rate.

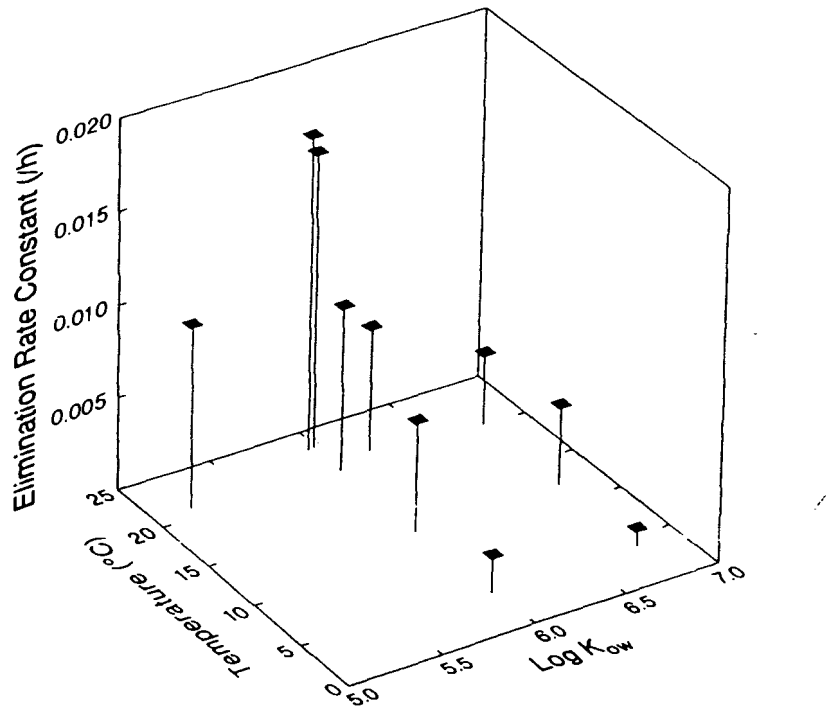

Figure 7. Changes in elimination rate constant ($k_{d\ in\ h}$) for a series of hydrophobic xenobiotics as a function of temperature (T in °C) and log octanol:water partition coefficient (log K_{ow}) in *Dreissena polymorpha*. k_d = 0.025 (±0.14) − 0.004 (±0.002) log K_{ow} + 0.00045 (±0.00015) T; r^2 = 0.059, p <0.01, n = 12 (value in parentheses is ±S.E.).

Direct correlation of filtering rate and particle concentration as seen in this study may be due, in part, to the condition of the organisms. Experiments were performed after mussels had been in the laboratory for several weeks. When animals were preexposed to *Chlorella* concentrations of 50 mg/L for 24 hr prior to initiating the filtration study, the filtration rate declined. However, the rate was still higher than previously found for zebra mussels (Ten Winkel and Davids, 1982). A second potential reason for the high filtration rates is that *Chlorella* is not the preferred food and may be deficient in some required nutrients.

The influence of temperature on filtering rate in zebra mussels has been carefully studied with two contrasting findings. Several authors report that filtering rate increases linearly with a rise in temperature within the 8–25°C range in both *Dreissena* and *Mytilus* (Schulte, 1975; Ali, 1970; Stanczykowska, 1977). Others report a temperature optimum of 12.5–15.0°C for filtering with a marked decline in filtering capacity on either side of the optimum (Theede, 1963; Walz, 1978). Although filtering rate was not measured as a function of temperature in the current study, two closely related functions — oxygen consumption and contaminant uptake — showed a steady increase in response to increasing temperature (Tables 1 and 3). These results

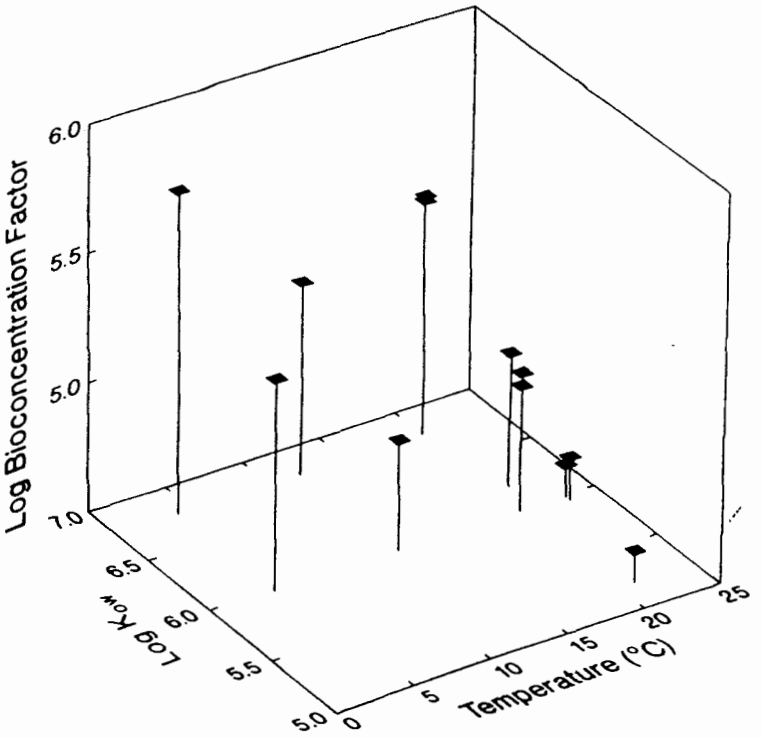

Figure 8. Dependence of log bioconcentration factor (log BCF) on temperature (T in °C) and log octanol:water partition coefficient (log K_{ow}) in *Dreissena polymorpha*. Log BCF = 2.4 ± 0.7 + 0.5 ± 0.1 log K_{ow} − 0.023 ± 0.007 T; r^2 = 0.79, p <0.001, n = 12 (value in parentheses is ±S.E.).

agree with studies on *Mytilus* which show that increased oxygen consumption and other physiological functions were related to increases in temperature. For example, Boryslawskj et al. (1985) state that the initial response of *Mytilus* to an increase in temperature is an observable elevation in the rate of ciliary beating. This activity increases the rate at which water is transported across gills, and thereby accentuates exposure of gill tissue to dissolved oxygen. If an increase in ciliary beating is the initial response to elevated temperature, this would also provide a mechanistic explanation for the observed increase in filtering activity with temperature: namely, cilia which line the incurrent siphon may beat more rapidly as temperature goes up; this, in turn, raises the amount of particulate material coming into the digestive system. The inverse relationship between mussel size and oxygen consumption agree with studies on *Mytilus* (Figure 3). In *Mytilus*, smaller mussels cleared more oxygen from water per milligram of tissue than did larger animals (Boryslawskj et al., 1985).

Comparing clearances for oxygen and contaminants, oxygen is accumulated much less efficiently than organic contaminants (Tables 1 and 3). The uptake rate of contaminants is similar to the filtration rate. Thus, one

can speculate that high filtration rates were not related to oxygen requirements, but to obtain adequate food resources. Because of high filtration rates, the accumulation of nonpolar organic contaminants is rapid as described as follows.

Toxicokinetics of Xenobiotics Compared to *Mytilus*

Accumulation of neutral lipophilic chemicals (log K_{ow} 3–6) in *Mytilus* is generally described as rapid (Lee et al., 1972; Clark and Finley, 1975; Nunes and Benville, 1979; Geyer et al., 1982; Widdows et al., 1983; Broman and Ganning, 1986) with significant body burdens being achieved within a few hours of exposure. For the most part, accumulation studies performed with *Mytilus* have involved steady-state analyses; that is, BCF or BAF values were determined from concentrations in mussels at steady state divided by the average concentration of chemical in the water. These studies indicate there is a fairly rapid approach to steady state, frequently within 10–30 days (Geyer et al., 1982; Hartley and Johnston, 1983).

In uptake experiments with zebra mussels, clearance of hydrophobic contaminants from water was rapid and linear throughout the 6-hr uptake phase (Table 2). There was no evidence of an approach to steady state or a leveling off of the uptake curve as has been reported for experiments conducted for similar duration with *Mytilus* (Ernst, 1977; Geyer et al., 1982). This may be attributable to the fact that the soft tissue of *Dreissena* consists of about 11.4% lipid (Walz, 1979) while the lipid content of *Mytilus* is less than 2% (Renberg et al., 1986). Since these materials are preferentially deposited in lipid-rich tissues, zebra mussels should accumulate significantly greater levels of contaminants.

Elimination of xenobiotics from contaminated mussels was slow and monophasic in nature (Table 2). Relatively slow k_d values have been reported for *Mytilus* and other bivalves when exposed to PCBs and PAHs (Kannan et al., 1989; Solbakken et al., 1982; Doherty, 1990; Ingebrigsten et al., 1988; Pruell et al., 1986); however, quantitative comparisons of k_d values cannot be made because of the different methods used. *Mytilus*, which has been most intensively studied, shows considerable variation in depuration activity among different studies. Elimination is frequently biphasic with an initial rapid phase followed by a second slower phase. This pattern has been found for a variety of chemicals including PCBs and PAHs (Broman and Ganning, 1986; Adema and Compaan, 1975; Hansen et al., 1978; Widdows et al., 1983). On the other hand, most investigators have found that depuration is monophasic and that the rate depends on the lipophilicity of the chemical (Clark and Finley, 1975; Pruell et al., 1986; Lee et al., 1972; Dunn and Stich, 1976; Hawker and Connel, 1986) and the lipid content of the organism (Landrum, 1988). When rapid depuration was seen, a low-level but persistent residue was re-

tained following elimination of 75–90% of the maximum body burden (Clark and Finley, 1975; Lee et al., 1972). Depuration in these cases may thus actually be biphasic in nature with the apparent retention of low-level residues representing the second, slower phase. This viewpoint is substantiated by studies in which the distribution of contaminants among different tissues was analyzed in *Mytilus*. Elimination varied considerably by tissue type; tissues with relatively high lipid levels eliminated xenobiotics at lower rates than lipid-poor tissues (Widdows et al. 1983). The initial rapid elimination noted in *Mytilus*, when up to 90% of contaminants is eliminated, represents elimination from organs with low lipid contents while the slower, long-term depuration reflects elimination from lipid reserves. The route of exposure may also play an important role in this determination. For example, Clark and Finley (1975) found that when petroleum hydrocarbons were absorbed directly across the gill, elimination occurred relatively quickly when animals were placed in clean water. However, when the same compounds were assimilated from food, absorbed across the gut, and stored in the hepatopancreas, elimination was much slower.

Although it is difficult to compare kinetic parameters, the potential for bioconcentration appears to be greater for *Dreissena* than for *Mytilus*. Renberg et al. (1986) reported a concentration factor of 13,000 for trichlorobiphenyl in *Mytilus*. Further, BAFs for a series of chemicals with log K_{ow} values between 5 and 6 ranged from 2,940 to 49,600 (Geyer et al., 1982). For a series of compounds with similar log K_{ow} values, BCFs in *Dreissena* varied from a low of 41,750–268,250 (Table 2). Thus, the potential for concentration of hydrophobic compounds in zebra mussels appears to be about an order of magnitude higher than for *Mytilus*. Again, this may be directly related to the difference in lipid content of the two species. As noted earlier, the lipid content of *Dreissena* is nearly an order of magnitude higher than the lipid content of *Mytilus*. As found in *Mytilus* (Widdows et al., 1983), a linear relationship was apparent between log K_{ow} and BCF in *Dreissena* (Figure 8).

Previous studies with *Mytilus* clearly indicate the importance of lipid content when determining kinetic rate constants as well as projected steady-state values. Lipid levels and, therefore, accumulation rates of individual chemicals vary significantly over a seasonal period. Hansen et al. (1978) estimated that changes in lipid content accounted for 18–32% of the variation in accumulation between individual mussels. Similarly, PCB content in *Mytilus* exposed to environmental PCBs varied in response to changes in lipid levels; in addition, the attainment of steady-state widely reported in other studies with *Mytilus* were only apparent since BCFs would change as a function of lipid metabolism (McDowell-Capuzzo et al., 1989). Contaminants accumulated by *Mytilus* were preferentially retained in lipid-rich tissues (Lee et al., 1972; Renberg et al., 1986; Mattson et al., 1988; Ingebrigsten et al., 1988), thus substantiating the hypothesis that lipid content is an important parameter of accumulation in *Mytilus*.

When the kinetic parameters from GLERL and OSU experiments are compared to each other, the uptake clearance and elimination rate constants were consistently lower in OSU experiments (Table 2). This was particularly noticeable for HCBP where the k_u values measured were 167 and 1073 mL/gWW/hr for OSU and GLERL experiments, respectively. The k_u for HCBP is believed to be artificially low in OSU experiments. Mussels used in the OSU-HCBP uptake experiment had been maintained in culture for nearly 2 months, during which time a protocol for culturing the mussels was still being developed. The feeding regime being used at the time was inadequate to maintain the mussels, and weight loss was observed among stock culture. Since much of the weight loss probably involved lipid mobilization, it is not surprising that k_u for OSU experiments was very low. For DDT, the k_u value found in OSU experiments was lower than found in GLERL experiments. Since these experiments were all performed with freshly collected mussels, the difference may be a function of size (Table 2). Mussels in OSU experiments were approximately 5–10 mm larger than those used in GLERL experiments. Larger animals can be expected to show lower uptake clearance rates than smaller animals (Figures 4 and 5). The use of different kinetic models may also account for part of the difference between kinetic measurements for the two experimental populations.

In zebra mussels, an increase in temperature had the effect of increasing uptake clearance and elimination rates for BaP and HCBP (Table 3, Figures 4 and 5). The increase in k_d was proportionately greater than the increase in k_u so that overall, BCF values declined with an increase in temperature. In multiple regressions of temperature and K_{ow}, and BCF, a significant negative correlation was demonstrated between temperature and BCF with the series of compounds used in regression analyses (Figure 8). Thus, the effect of temperature on toxicokinetic parameters does not appear to be limited to specific compounds. The greater effect of temperature upon elimination rate in comparison to uptake clearance has been demonstrated in other freshwater aquatic organisms such as the midge, *Chironomus riparius* (McIntyre, 1988; Lydy et al., 1990).

In *Mytilus*, temperature exerts a clear influence on contaminant accumulation; an increase in temperature leads to an increase in accumulation rates (Boryslawskj et al., 1985). In this study, when environmental temperature was increased from 5 to 15°C, accumulation of petroleum hydrocarbons increased significantly. Elimination rate constants were also seen to be responsive to changing temperature although the two phases of the biphasic elimination curve were affected differently. The rate constant for the initial rapid phase of depuration increased linearly with temperature. However, the second phase of depuration was temperature independent.

Higher filtration and respiratory rates were measured for smaller than large zebra mussels (Figure 3). Smaller mussels also showed relatively greater

uptake clearance rates for BaP and HCBP (Figures 4 and 5). Because k_d was not analyzed as a function of mass, it is not clear whether projected steady-state values would increase or decrease in smaller mussels. Animals which feed selectively on zebra mussels within a smaller size range may be exposed to increased levels of contaminants if accumulation is skewed toward smaller mass mussels.

Muncaster et al. (1990) reported that size was negatively correlated with levels of accumulation in two freshwater mollusks. Melaouah (1990) demonstrated that accumulation of amino acids from water in mollusks was dependent upon larval size for which a negative correlation with size was observed. However, accumulation of the latter compounds may not be passive and, therefore, involve a different uptake mechanism (Swinehart and Cheng, 1987; Widdows et al., 1983). Several authors have argued that the higher filtration and respiratory rates measured in small mussels should also lead to increased accumulation of contaminants (Vahl, 1973; Bayne and Widdows, 1978).

Reasonably good correlations were found for kinetic parameters when regressed against temperature and K_{ow} (Figures 6, 7, and 8). The lipophilicity of the chemical, as expressed by log K_{ow}, is clearly an important determinant of uptake clearance rates (Figure 6), elimination rate constants (Figure 7), and BCF (Figure 8). Of particular note was the negative relationship of elimination rate constant to K_{ow} (Figure 7). This has also been reported for *Mytilus* (Hawker and Connel, 1986). Likewise, there are several reports of BCF values for neutral lipophilic contaminants increasing as a function of log K_{ow} (Ernst, 1977; Geyer et al., 1982), although Pruell et al. (1986) found that BCFs for PCBs were significantly higher than for PAHs possessing identical log K_{ow} values. In general, results of the current study on *Dreissena* and the literature reports of *Mytilus* confirm the predictive value of log K_{ow} in forecasting levels of bioaccumulation under laboratory conditions.

Impact on Contaminant Cycling

Kinetic experiments conducted in these studies have used water as the primary medium from which contaminants were sorbed. However, studies with *Mytilus* have shown that significant accumulation of hydrophobic xenobiotics can also occur from food (Clark and Finley, 1975; Arapis et al., 1984) and sediment (Dame and Dankers, 1988; Pruell et al., 1986; Doherty, 1990). In OSU experiments, preliminary attempts were made to measure the relative rates of accumulation of HCBP from water, rates of accumulation from water in the presence of an algal food source, and rates from the assimilation of contaminated algae. When uncontaminated algae were fed to zebra mussels in contaminated water, k_u was reduced threefold while elimination was increased by a factor of 12 (Table 4). From this result, it can be inferred

TABLE 4. Influence of Route of Exposure in HCBP Clearance and Elimination in *Dreissena polymorpha* in OSU Experiments

Exposure Route	k_u[a] (mL/g/hr)	k_d[a] (/hr)	BCF	$t_{1/2}$ (hr)
Water (no food) (n = 30)	167 (81)	0.004 (0.001)	41,750	173
Water (with food) (n = 30)	59 (16)	0.055 (0.009)	1,072	13
Algae (clean water) (n = 60)	7 (3)	0.03 (0.02)	233	23

[a] Values given are means with standard deviations in parentheses.

that the uptake of HCBP may have been impeded by sorption of HCBP to algae, thereby reducing its availability for uptake. Uptake measured in this experiment potentially reflects accumulation from both food and water but, since the binding of HCBP to algal cells was not measured in this experiment, definitive conclusions are precluded. The order of magnitude increase in k_d when uncontaminated algae was present in the water suggests that elimination of HCBP from zebra mussel tissues is enhanced by the presence of a sorbent in the gut of the mussel. The combination of lower uptake and increased elimination led to a marked reduction in BCF and $t_{1/2}$ for HCBP (Table 4). Our data also indicate that contaminants can be readily assimilated from food into mussels, although uptake clearance rates for this route were significantly lower than for the other two routes. Because assimilation from contaminated food is slow, it is likely that this route did not contribute significantly to the body burden of mussels exposed for 6 hr to contaminated water in the presence of *Chlorella*. Although accumulation from food is comparatively slow, it may be a significant route of accumulation in nature especially when mussels settle in benthic environments. Hydrophobic contaminants which reach the benthic zone will most likely be sorbed to organic materials including algae, dissolved organic carbon, and sediment. The ability of zebra mussels to assimilate contaminants from these media must be considered in order to fully understand contaminant accumulation. The relative affinity of compounds for each medium as well as dynamic processes such as lipid metabolism, temperature, and phytoplankton abundance will ultimately determine the complex equilibria between all compartments.

One potentially important issue which must be considered is whether zebra mussels can restructure contaminant cycling by removing contaminants from the pelagic zone and concentrating them in the benthic environment. Filtered material which is not assimilated is compacted into a pellet, wrapped with a mucous coating, and deposited on the bottom as pseudofeces. Benthic organisms may be exposed to highly concentrated hydrophobic xenobiotics if these organically rich pseudofeces are subsequently consumed.

The concern for benthos is not academic. Based on some rough calculations, the collective filtering capacity of the mussels is relatively large. If it is assumed that a single mussel can filter 61 mL/hr, then each animal will filter a volume of 1.46 L/day. If it is further assumed that zebra mussels are present in densities of 10,000/m^2 (which is conservative) and that they cover 1% of the lake, it would take 24 and 52 days for the mussels to completely filter Lake St. Clair and the western basin of Lake Erie, respectively. If a more realistic mussel density of 50,000/m^2 was assumed, the filtering time is reduced to 10 days for both lakes. Clearly, the mussels can potentially exert a major influence on phytoplankton distributions in these lakes. If mussels filter contaminated material (either water or food), then the opportunity for redirecting contaminant distributions in these lakes also exists. Furthermore, the finding that assimilation of HCBP from contaminated *Chlorella* was slow implies that HCBP may remain in unassimilated algal cells in significant amounts. The deposition of this pollutant in pseudofeces or feces is highly probable. Gauging the impact of the zebra mussels on the Great Lakes must therefore include an assessment of where these compounds are going and whether alterations in patterns of contaminant cycling are occurring.

SUMMARY

Using both mass-based and concentration-based kinetic models, *Dreissena polymorpha* was shown to rapidly accumulate substantial body burdens of PCBs and PAHs. Differences in kinetic parameters between two experimental populations of mussels were attributable primarily to mussel length. Smaller mussels showed much higher uptake clearance rates than did larger mussels. Environmental temperature profoundly altered uptake clearance and elimination rates such that total accumulation was significantly greater at lower temperatures. The relatively high lipid content of zebra mussels probably accounts for accentuated accumulation of contaminants relative to *Mytilus*.

It is clear that zebra mussels can accumulate hydrophobic chemicals from several sources and that the levels of accumulation will be comparatively high. In feeding experiments, zebra mussels assimilated HCBP from contaminated algae even though at a much lower rate than absorption from water. These preliminary experiments showed that accumulation from food as well as from water will occur in nature. The impact of zebra mussels on contaminant cycling is believed to be potentially important in the Great Lakes.

ACKNOWLEDGMENTS

GLERL contribution number 748 is appreciated. The OSU component of this research was supported in part by the Ohio Sea Grant College Program, project R/MR-4 under grant NA89AA-D-SG B2 with additional support from the Ohio State University and the Ohio Board of Regents.

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ZEBRA MUSSELS

BIOLOGY, IMPACTS, AND CONTROL

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Donald W. Schloesser

LEWIS PUBLISHERS
Boca Raton Ann Arbor London Tokyo

Front Cover. Zebra mussels attached to a reed taken from Lake Maarsseveen in the Netherlands. (Photograph by Simon van Mechelen and courtesy of Michiel Kraak, University of Amsterdam, The Netherlands.)

Library of Congress Cataloging-in-Publication Data

Zebra mussels : biology, impacts, and control / edited by Thomas F. Nalepa and Don Schloesser.
p. cm.
Includes bibliographical references and index.
ISBN 0-87371-696-5
1. Zebra mussel. 2. Zebra mussel--Control. 3. Zebra mussel--Control--Environmental aspects. I. Nalepa, T. F. II. Schloesser, Donald W.
QL430.7.D8Z45 1992
594'.11--dc20 92-10333
CIP

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

PRINTED IN THE UNITED STATES OF AMERICA
2 3 4 5 6 7 8 9 0

Printed on acid-free paper